

## Regulation of Water Permeability of the Skin of the Treefrog, *Hyla arborea japonica*

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**ABSTRACT**—Water permeability of excised skin was investigated in Japanese treefrogs during the non-breeding season. In normal frogs, water flowed inward across the ventral skin at a rate of  $0.30 \mu\text{l}/\text{cm}^2/\text{min}$ , and outward at a rate of  $0.0015 \mu\text{l}/\text{cm}^2/\text{min}$  across the dorsal skin. The water flux across the ventral skin was greatly stimulated when frogs were submitted to dehydration. The sympathomimetic agent noradrenaline and the beta-adrenergic receptor agonist isoproterenol (IP) greatly increased the water flux across the ventral skin, but these had no effect on the dorsal skin. The beta-receptor antagonist propranolol (PP) suppressed the enhanced water influx by dehydration or IP treatment. However, PP had no effect on normal water permeation across the ventral skin. Ouabain, a specific inhibitor of  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, also suppressed the stimulatory effect of IP, whereas this inhibitor again had no effect in normal water flux of the ventral skin. The adrenergic alpha-receptor agonist phenylephrine had no effects on water permeation in either ventral or dorsal skin. Prolactin administration for one week prior to the experimentation decreased water permeability significantly across the ventral skin in both normal and stimulated preparations. These results all argue for the presence of two types of water transport system in the ventral skin of the Japanese treefrog. The first is mediated by the adrenergic beta-receptor and by  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity and may be activated by dehydration. The second is not mediated by the adrenergic receptor and may function as the normal, basal water permeation.

### INTRODUCTION

Anuran amphibians are known not to take water orally. They take the most of their water through the skin [1-6]. Water permeation through the skin is mainly confined to the pelvic patch [7-9] and can be modified by various factors, including sympathomimetic agents [10-15] and neurohypophysial hormones [1, 2, 5, 7, 15]. Water permeation in the skin is stimulated by dehydration [4, 11]. Water permeability differs according to both species and season. Generally, terrestrially-adopted species have more permeable skin than do aquatic species [8, 9]. Water also evaporates readily from normal frog skin [1, 3]. This evaporation helps to cool the animal, and is known to be less in terrestrially-adopted anurans such as toads and treefrogs [16, 17]. These findings raise the para-

doxical question of how these anurans manage to survive in hot and dry environments, where they cool themselves at the cost of water which is already scarce in their habitats.

The Japanese treefrogs are known to have survived long periods of drought, and have been reported to accumulate glycosaminoglycans in their epidermis following dehydration treatment. The glycosaminoglycans might cause retardation of water movement across the skin [18]. Prolactin administration brought similar morphological changes in the skin of these frogs. In the present experiment we intended to find out regulating factors of water permeation in the Japanese treefrogs, especially in terms of neural and hormonal control under the dehydrated condition.

### MATERIALS AND METHODS

Japanese treefrogs, *Hyla arborea japonica*, were collected from fields and kept for at least one week in a terrarium in the laboratory prior to the

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experiments. The terrarium was maintained at 24°C under 12L-12D photoperiod. Frogs were fed periodically with crickets and allowed access to water *ad libitum*. Young adults weighing  $1 \pm 0.3$  g were used. In these specimens no sexual difference was observed in water permeation between the period November through February during which time the present experiment was performed.

Water permeation of the skin was measured by recording water movement between two small chambers partitioned with an experimental skin. Both chambers were connected with a filter holder (Swinnex sx00 013 00, Millipore Corp.), on which excised skin was mounted in the position of a filter. The chamber facing the dermal side (the inside) of the skin was filled with an airated, modified Ringer solution (230 mOsM) [19] and attached with a graded pipette. The chamber facing the epidermal side was filled with distilled water (DW). Net water movement across the partitioned skin was measured by recording changes of the meniscus in the pipette. Dilute Ringer solution (20 mOsM) was substituted for the DW in the outside bath, but significant difference in water movement was not detected. Thus, DW was used in all experiments for the outside bath. The osmotic difference between the two chambers was maintained at approximately 230 mOsM throughout the experiments.

To dehydrate frogs, they were kept in a chamber in which relative humidity was maintained at 90%. The formation of water droplets in the chamber was carefully prevented. Frogs were neither fed nor allowed to access to water during this treatment. Dehydration treatment ended after approximately 40 hr when the animal lost 30% of its initial weight.

In the prolactin treatment, frogs were injected daily with ovine prolactin (Sigma, 0.25 IU (8.1  $\mu$ g)/gbw/day) in 2 ml of Ringer solution intraperitoneally for 7 days. During this treatment they were kept under the normal conditions and fed as per usual. As a control, another group of frogs were injected with egg albumin (Nakarai Chem. LTD) in the same protein concentration as prolactin.

Five animals were used in each experiment and the experiment was repeated three times. Since

the available filtrating area of the apparatus was  $0.75 \text{ cm}^2$ , direct reading of the pipette was calibrated as a net water movement in  $\mu\text{l}/\text{cm}^2$  and plotted in 10 min intervals for 100 min. In order to compare water permeability of the experiments, an average water flux (Jw) was calculated and expressed in  $\mu\text{l}/\text{cm}^2/\text{min}$ . Positive value in Jw shows water movement from the epidermal side to the dermal side and a negative value indicates the reverse.

## RESULTS

*Hyla arborea japonica* exhibited a fundamental difference between water permeation of the dorsal and ventral sides of their isolated skin (Fig. 1). Under normal conditions, that is, immersing the dermal side of the excised skin in Ringer solution (230 mOsM) and the epidermal side in distilled water, water flowed inwardly across the ventral skin at a constant rate of  $0.29 \mu\text{l}$  per  $\text{cm}^2$  per min, or  $Jw = +0.29$ . In the dorsal skin, however, water permeation took place at a lower rate of  $Jw = -0.015$ , which meant water movement from the inside to the outside. The movement was against the osmotic gradient.

When frogs were kept in the dehydration chamber (24°C, 90% relative humidity) for approximately 40 hr and had lost nearly 30% of their initial weight, the ventral skin enhanced water permeability (Fig. 1). The water flux was not constant throughout the experiment. It lessened over time, but the average rate over 100 min was as high as  $Jw = +0.91$ , four times greater than that in the control group. In dorsal skin, however, no such change in water permeability was observed during dehydration treatment.

When noradrenalin, one of the sympathomimetic agents, was added to the inside bath of the normal preparation, the water flux increased to  $Jw = 1.52$  in ventral skin, five times the control rate (Fig. 2). Daily administration of prolactin for 7 days caused a significant decrease in water permeation across the ventral skin (Fig. 2). The water flux,  $Jw = +0.08$ , was only one-fourth the control value. Dehydration had a less pronounced effect in the frogs pre-treated with prolactin. The rate of water flux ( $Jw = +0.51$ ) was less than 60% of that

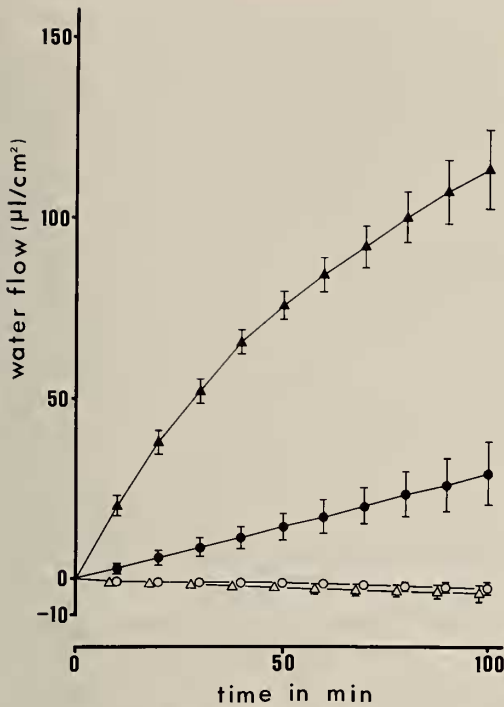


FIG. 1. Rates of the water permeation across the excised skin of the treefrog. Water flowed from mucousa to serosa at a rate of  $29 \mu\text{l}/100 \text{ min}$  across the normal ventral skin (filled circle) and at a rate of  $-1.5 \mu\text{l}/100 \text{ min}$  across the dorsal skin, which showed a net water movement from the inside to the outside (open circle). Dehydration of the frogs caused a marked increase in the water flow across the ventral skin, to a rate of  $112 \mu\text{l}/100 \text{ min}$  (filled triangle), but dehydration had no effects on the dorsal skin (open triangle). The vertical bar shows the standard margin of error.

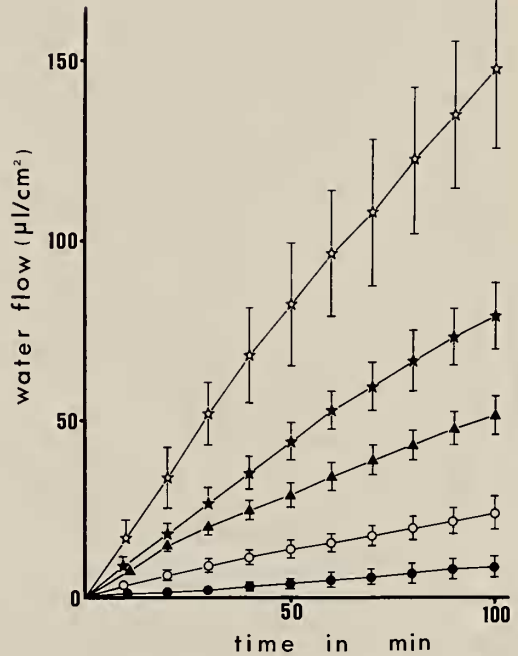


FIG. 2. Hormonal effects on water permeation across the excised ventral skin of the treefrog. The sympathomimetic agent noradrenalin ( $10^{-5} \text{ M}$ ) added to the inner experimental medium caused an enhancement on the water flow (open star). Daily administration of prolactin ( $8.1 \mu\text{g}/\text{day}$ ) for a week decreased the water flow (filled circle). Prolactin pretreatment also suppressed the water flow which had previously been stimulated by dehydration (filled triangle) and by noradrenalin administration (filled star). Albumin administration serving as the control for prolactin-treated group, caused no effects on water flow (open circle). The vertical bar shows the standard margin of error.

of dehydrated normal frogs (Fig. 2). Stimulation of water flux by noradrenalin was also significantly depressed in the prolactin-treated frogs. The water flux ( $J_w = +0.78$ ) was one-half of the normal skin treated with noradrenalin. When egg albumin at the same protein concentration as the prolactin was injected for the same term, no significant change was observed (Fig. 2).

An adrenergic beta-receptor agonist, isoproterenol (IP,  $10^{-5} \text{ M}$ ), enhanced the water flux ( $J_w = +1.6$ ) similarly to the noradrenalin treatment. Propranolol (PP,  $10^{-5} \text{ M}$ ), an adrenergic beta-receptor antagonist, not only counteracted isoproterenol, but also depressed the water flux induced by the

dehydration significantly to  $J_w = +0.61$ , two-thirds the rate of dehydrated frogs (Fig. 3). However, propranolol did not affect the water flux in normal frogs at all. Isoproterenol administration to the normal preparation caused an instantaneous rise of the water flux to  $J_w = +1.6$  (Fig. 3). However, replacement of this medium by one containing propranolol caused slow and gradual depression of the water flux, which finally reached the normal rate,  $J_w = +0.35$ , in 40 min (Fig. 3). Alpha-adrenergic agents such as phenylephrine (alpha-receptor agonist) or dibenamine (alpha-receptor

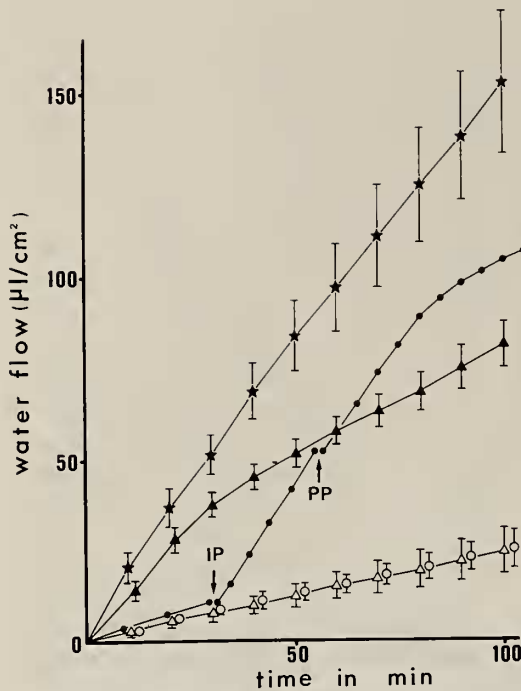


FIG. 3. Effects of adrenergic agents on water permeation across the ventral skin of the treefrog. When the adrenergic beta-receptor agonist isoproterenol ( $10^{-5}$  M) was added to the experimental medium, the water flux increased markedly (filled star), while the addition of the alpha-agonist phenylephrine ( $10^{-5}$  M) had no effect at all (open circle). An adrenergic beta-antagonist propranolol (PP,  $10^{-5}$  M) counteracted the isoproterenol stimulation (IP). Propranolol suppressed the enhancement induced by dehydration (filled triangle). Propranolol, however, had no effect on normal skin (open triangle). The vertical bar indicates the standard margin of error.

antagonist) had no effect on the water flow across the skin of either dehydrated or normal frogs.

Stimulation of the water flux by isoproterenol was very strong. It persisted continuously for 60 min or more even after the agent had been washed out from the medium (Fig. 4). However, pretreatment of the skin with ouabain, a specific  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase inhibitor, obstructed the stimulating effect of isoproterenol completely. Ouabain, however, did not affect the basal water flux in the normal frogs.

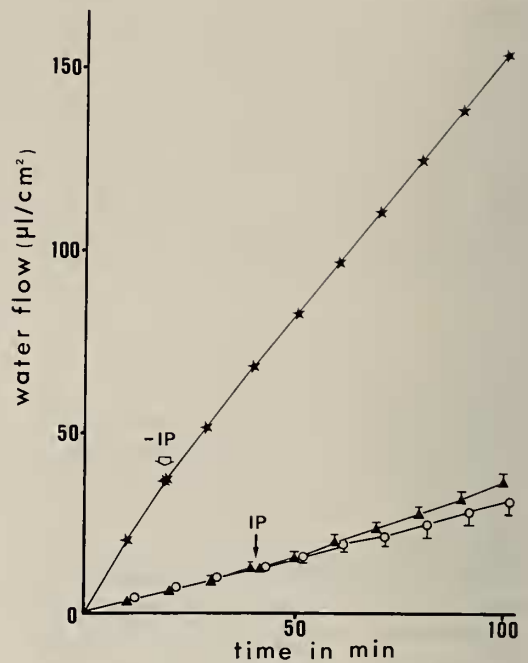


FIG. 4. Effect of ouabain on the isoproterenol-stimulated water flow across the ventral skin of the treefrog. Stimulation by isoproterenol for 20 min caused a prolonged increase in the water flow (filled star) even after the agent had been removed (-IP). However, pretreatment with ouabain ( $10^{-5}$  M) for 40 min completely counteracted the stimulatory effect of isoproterenol (filled triangle). Ouabain treatment, however, did not have any effect on normal skin (open circle).

## DISCUSSION

It has previously been reported in American treefrogs that water flux in the ventral skin is 10 to 20 times greater than that of the dorsal skin [8]. The present study shows an even more remarkable dorso-ventral difference in water permeation across the skin of Japanese treefrogs. When excised skin was examined, water normally flowed inwardly in great quantities across the ventral skin, while it flowed outwardly in a small amount across the dorsal skin. When the treefrogs were kept in dehydrated conditions, water flux increased markedly in the ventral skin, but no significant change was observed in the dorsal skin. Similar increase in water flux can be induced by adrenergic beta-stimulation in various anurans [10-15].



When dehydrated treefrogs, which had lost 30% of their body weight, were allowed to take water, they recovered 90% of their previous weight within 10 min by absorbing the water across the ventral skin (unpublished data). This rapid water absorption across the ventral skin seems to be regulated by the adrenergic beta-receptor, since propranolol, a beta-receptor antagonist, depressed enhancement of the water flux caused by dehydration. In the present experiment, the propranolol depressed only the stimulated water flux across ventral skin, and did not affect normal water flux across either side of the skin. Thus, it is probable that there are two types of water pathways in the ventral side of the treefrog skin. The first pathway is mediated by beta-receptor stimulation that begins to function in cases of urgent water requirement, such as dehydration, and is ouabain sensitive. The second pathway is the basal one which functions under the normal condition and is not affected by beta-adrenoceptor stimulation. Similarly, two water transport systems have also been reported in toads [10, 14]. In one of these studies [14] alpha-receptors have been reported to inhibit water flux. However, no effects of alpha-agents on the water flow across the ventral skin of Japanese treefrogs were observed in this study.

De Sousa *et al.* reported that ouabain did not have any effect on the isoproterenol-stimulated water flow in toad skin [10]. In the present experiment, concomitant administration of ouabain and isoproterenol also failed to produce any clear suppressive effect. This may be due to the difference in modes of action of the two agents. As mentioned above, the stimulating effect of isoproterenol appears instantaneously and persists for a certain period even after it has been washed out, while ouabain takes 30 to 40 min to produce the suppressive effect. Therefore, it is likely that the  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase activity is necessary for the attainment of the beta-action.

Pretreatment with prolactin for one week reduced the water flux approximately 50% in the ventral skin. Suppression by prolactin occurred not only in the stimulated water flow but also with the basal flow in the normal frogs. This prolactin suppression seems to be caused by a mechanism different from that of the suppressive agents men-

tioned above. Prolactin is generally known to prevent osmotic water permeation in adult urodiles [5, 20], as well as in larval anurans [20] and fresh water fish [19]. This is primarily due to the mucous secretion on the integument under the hormonal stimulation. The mucous not only functions as the water-resistant coating of the skin, but may also produce certain osmotic effects itself by retaining electrolytes in the coating, resulting in a lesser osmotic gradient between inside and outside of the animal body [18]. Indeed, frogs have been reported to have increasing amount of prolactin receptor in the skin during the breeding season when they become aquatic [21]. Suppression of water flux by prolactin pretreatment in the present experiment also seems to be related to the mucous secretion, which has previously been observed histochemically in those frogs (not published). On the other hand, extirpation of pars distalis or removal of the whole pituitary in the toad *Bufo bufo* depressed water flux which had been stimulated by dehydration [22]. This, in a sense, conflicts with the present findings that administration of prolactin depressed the stimulated water flow. However, the hypophysectomy does not indicate disappearance of prolactin alone in the animals.

In summary, the following results are characteristic of this species: marked difference in dorsal and ventral water permeation, particularly, reverse-directional water movement in ventral and dorsal skin under normal conditions, and two water pathways in the ventral skin, including a regulatory system mediated by adrenergic beta-receptor and  $\text{Na}^+$ ,  $\text{K}^+$ -ATPase, and a basal system indifferent to these mediators. Both pathways are regulated by prolactin.

## REFERENCES

- 1 Deyrup, I. J. (1964) Water balance and kidney. In "Physiology of the Amphibia". Ed. by J. A. Moor, Academic Press, New York, pp. 251-328.
- 2 Shoemaker, V. H. and Nagy, K. A. (1977) Osmoregulation in amphibians and reptiles. *Ann. Rev. Physiol.*, **39**: 449-471.
- 3 Deullman, W. E. and Trueb, L. (1986) *Biology of Amphibians*. McGraw-Hill, New York, pp. 197-228.
- 4 Bentley, P. J. and Yorio, T. (1979) Do frogs drink?

- J. Exp. Biol., **79**: 41-46.
- 5 Lorenz, C. A. and Bern, H. A. (1982) Prolactin and osmoregulation in vertebrates. *Neuroendocrinology*, **35**: 292-304.
  - 6 Bentley, P. J. (1982) *Comparative Vertebrate Endocrinology*. 2nd. Ed., Cambridge Univ. Press, Cambridge, pp. 303-337.
  - 7 Hillyard, S. D. (1976) Variation in the effects of antidiuretic hormone on the isolated skin of the toad, *Scaphiopus couchi*. *J. Exp. Zool.*, **195**: 199-206.
  - 8 Yorio, T. and Bentley, P. J. (1977) Asymmetrical permeability of the integument of treefrogs (*Hyllidae*). *J. Exp. Biol.*, **67**: 197-204.
  - 9 McClanahan, L. and Baldwin, R. (1969) Rate of water uptake through the integument of the desert toad, *Bufo punctatus*. *Comp. Biochem. Physiol.*, **28**: 381-389.
  - 10 De Sousa, R. C. and Grosso, A. (1982) Osmotic water flow across the abdominal skin of the toad *Bufo marinus*: effect of vasopressin and isoprenaline. *J. Physiol.*, **329**: 281-296.
  - 11 Yokota, S. D. and Hillman, S. S. (1984) Adrenergic control of the anuran cutaneous hydroosmotic response. *Gen. Comp. Endocrinol.*, **53**: 309-314.
  - 12 Brown, D., Grosso, A. and de Sousa, R. C. (1980) Isoproterenol-induced intramembrane particle aggregation and water flux in toad epidermis. *Biochem. Biophys. Acta*, **595**: 158-164.
  - 13 Hyllard, S. D. (1979) The effect of isoproterenol on the anuran water balance response. *Comp. Biochem. Physiol.*, **62C**: 93-95.
  - 14 Gamundi, S. S., Scheucher, A. and Coviello, A. (1986) Alpha-2 adrenergic agonists inhibit basal and stimulated osmotic water permeability in toad skin. *Comp. Biochem. Physiol.*, **84C**: 199-203.
  - 15 Elliot, A. B. (1968) Effect of adrenaline on water uptake in *Bufo*. *J. Physiol.*, **197**: 87-88.
  - 16 Wygoda, M. L. (1984) Low cutaneous evaporative water loss in arboreal frogs. *Physiol. Zool.*, **57**: 329-337.
  - 17 Withers, P. C., Hillman, S. S., Drewes, and Sokol, O. M. (1982) Nitrogen excretion in sharp-nosed reed frogs (*Hyperolius nasutus*: Anura, Hyperoliidae). *J. Exp. Biol.*, **97**: 335-343.
  - 18 Shephard, K. I. (1981) The influence of mucus on the diffusion of water across fish epidermis. *Physiol. Zool.*, **54**: 224-229.
  - 19 Johnsen, A. H. and Nielsen, R. (1984) Correlation between cAMP in isolated frog skin epithelium and stimulation of sodium transport and osmotic water flow by antidiuretic hormone and phosphodiesterase inhibitor. *Gen. Comp. Endocrinol.*, **54**: 144-153.
  - 20 Loudi, B., Biciotti, M. and Viotto, B. (1982) Cutaneous osmoregulation in *Triturus cristatus carnifex* (Laur.) (Urodela). *Gen. Comp. Endocrinol.*, **46**: 452-457.
  - 21 D'Istria, Fasano, S. and Delrio, G. (1987) Prolactin receptors in the male *Rana esculenta*. *Gen. Comp. Endocrinol.*, **68**: 6-11.
  - 22 Cristensen, C. U. and Jorgensen, C. B. (1972) Role of pars distalis and pars nervosa of the hypophysis in the water economy of the toad. *Gen. Comp. Endocrinol.*, **18**: 169-174.